Experimental eutrophication on an intertidal sandflat: effects on microphytobenthos, meio- and macrofauna

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Abstract

A field experiment was carried out to simulate effects of eutrophication on the benthic community of a sandy tidal flat in the Wadden Sea. A new device, the pore-water manipulator, was used to enhance pore water concentrations of phosphate and ammonium during a period of 18 weeks. The microphytobenthos responded with a significant biomass increase which lasted during the entire experiment. The species composition changed and particularly cyanobacteria of the genus *Merismopedia* increased. The experiment indicated that the microphytobenthos was N-limited. Meio- and macrofauna showed no reaction on the increased microphytobenthos biomass. It is supposed that the food availability for the fauna did not improve because the groups of algae taking over are hardly grazed.

Keywords: eutrophication, microphytobenthos, cyanobacteria, zoobenthos, Wadden Sea.

Introduction

Large coastal areas of the North Sea are considered to be eutrophied waters. Numerous effects of eutrophication, e.g. massive algal blooms followed by anoxia on the bottom, have been reported from this region (Nelissen & Stefels 1988, and references therein). For the microphytobenthos, a doubling of biomass and production was recorded in the Dutch Wadden Sea from 1968 to 1981 (Cadée 1984). In the same period and region, Beukema & Cadée (1986) found a doubling of macrozoobenthos biomass, too. Relating these findings to the eutrophication of the North Sea, the following questions are raised:

1. In which way could eutrophication of the North Sea reach and affect the benthic communities in the Wadden Sea?
2. Are the primary producers, i.e. the microphytobenthos, limited by nutrients so that they could respond to an increased supply of nutrients?
3. Are the consumers, i.e. the meio- and macrozoobenthos, food-limited so that they could react to an increased supply of food?

These questions were investigated in this study simultaneously using a field experiment. To study the effect of increased concentrations of inorganic nutrients in the pore water, due to a simulated increased remineralization of organic matter in the sediment, experimental plots on a tidal flat were enriched by a new technique.

Similar approaches have been previously attempted for freshwater sediments (Pringle & Bowers 1984, Carrick & Lowe 1989), salt marshes (Sullivan & Daiber 1975, v. Raalte *et al.* 1976, Wiltse *et al.* 1984) and sublittoral sediments (Granéli & Sundbäck 1985). However, insufficient work has been carried out in the Wadden Sea to cast some light on these problems. Nevertheless, there are some indications from field investigations (Otte 1979, Höpner & Wonneberger 1985) that the microphytobenthos of this habitat is indeed sensitive to anthropogenic nutrient enhancement.

Materials and methods

Study site

The experiment was carried out on an intertidal sandflat in the 'Königshafen' (Figure 1), a sheltered bay on the island of Sylt, German Wadden Sea (55°01'N, 08°63'E). The mean tidal range is 1.7 m; the experimental plots emerge for 6-7 hours every ebb-tide. The sediment is coarse grained and well-sorted (median grain size = 323 µm). The oxygenated surface layer is 1 cm deep. The sediment community is dominated by the lugworm *Arenicola marina*. There are no macrophytes on the flat.

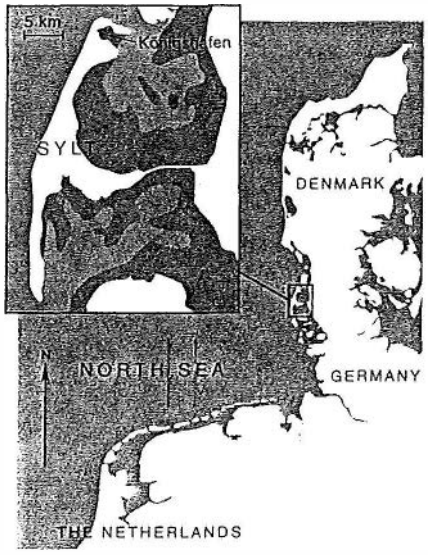

Figure 1.
The island of Sylt with Königshafen in the eastern part of the North Sea. Tidal flats are shaded.

Experimental set-up; the pore-water manipulator

For the experimental enrichment of the pore water with dissolved inorganic nutrients, a new device, the pore-water mani-

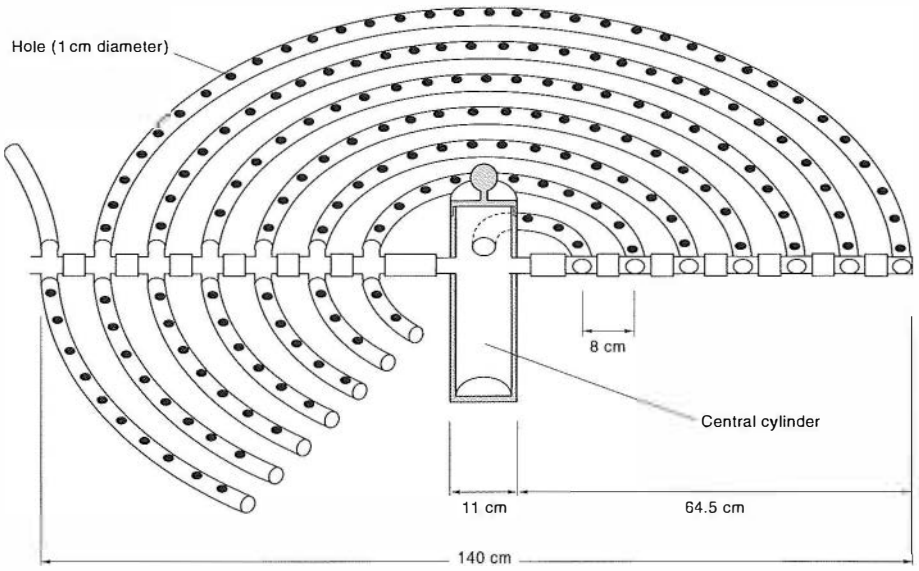

Figure 2.
The pore-water manipulator (PWM). The central cylinder and the pipe spirals are shown in longitudinal section (on left side at 45°).

pulator (PWM) was designed. The PWM was buried in the sediment, 8 cm below the surface. The PWM consists of two main elements (Figure 2):

1. A double spiral of flexible PVC pipes (inner diameter: 16 mm; outer diameter: 22 mm). The upper side is perforated by holes (diameter: 10 mm) placed at a distance of 50 mm from each other. To prevent sediment penetrating into the pipes, the holes are covered with gauze. The two spiral pipes wind in opposite directions with six whorls. In addition, two radial connection pipes run from the center of the spiral to the outermost part, fitted into the whorls by means of cross connections. The ends of the spirals reach above the sediment surface and are closed by a rubber stopper.
2. A central cylinder. This consists of a PVC tube with a length of 30 cm and an inner diameter of 9.5 cm. The cylinder is closed at both ends with a lid and is connected with the pipe spirals by means of four lateral openings. The cylinder is buried in the sediment in such a way that only its upper edge with the lid is above the sediment. Thus, the cylinder works as the connection between the sediment surface and the pipe spirals at 8 cm depth. The upper lid of the cylinder can be opened and, by a connecting piece, it is possible to fill the whole system with an enrichment solution. The enrichment was carried out at ebb-tide. The water column in the connecting piece (height: 30 cm) provides a symmetrical spreading of the enrichment solution through the cylinder, the pipe spirals and through the holes into the sediment by means of pressure. Thus, the sediment is soaked with the enrichment solution from below. The sampling area above a PWM is 1.6 m^2.

After burying the systems in the sediment, the plots were allowed to regenerate for three weeks. The experiments ran for four months (June-September 1989). The solutions were renewed once a week. The enrichment solution had the following concentrations of dissolved inorganic nutrients: (PO_4^{3-}): 1336 $\mu mol \cdot dm^{-3}$, (NO_2^-): 7 $\mu mol \cdot dm^{-3}$, (NO_3^-): 8168 $\mu mol \cdot dm^{-3}$, (NHO_4^+): 4033 $\mu mol \cdot dm^{-3}$, ($Si(OH)_4$): 21 $\mu mol \cdot dm^{-3}$.

Sampling

Samples for analysis of the pore water concentration of the dissolved inorganic nutrients (PO_4^{3-}, NO_2^-, NO_3^-, NH_4^+. $Si(OH)_4$) were taken several times with a specially designed soak syringe from each plot. The organic content of the sediment was determined as loss of ignition after 95 days. For the determination of the chlorophyll-*a* content, sediment samples from the upper 1 cm were taken once a week with a plastic corer (diameter: 11 mm, 3 subsamples from each plot). With the same corer, samples for counts of living autotrophic microflora were obtained on five (for diatoms) and on six (for cyanobacteria) occasions. Meio- and macrofauna were investigated for short-term reactions (after 20 days) and for long-term reactions (after 89 and 95 days, respectively). Samples were taken with plastic corers of different diameters to a sediment depth of 2 cm (meiofauna) and 8 cm (macrofauna). Two to three subsamples were taken from each plot and for each group.

Nutrient analysis

The samples for the analysis of dissolved, inorganic nutrients were – if necessary – diluted with distilled water and analysed immediately according to the methods described in Grasshoff *et al.* (1983).

Organic content
Sediment samples for the determination of the organic content were dried (24 h, 110 °C) and burnt (5 h, 540 °C).

Chlorophyll-a
Chlorophyll-*a* content was analysed by a modification of the Strickland & Parsons (1968) method after extraction with 100 % acetone (2 h) and 30 min of ultrasonication treatment.

Microalgal cells
Epipelic (= not attached) cells were swept into suspension, an aliquot of which was used for counting the cells in an inverted microscope. The episammic (= attached on sand grains) cells were detached from their substratum by ultrasonication for 12 min. To prevent cell breakage by overheating, this treatment was carried out in an ice bath. Cells were counted in a Bürker counting chamber using epifluorescence microscopy. Samples for cyanobacteria were diluted, ultrasonicated (5 min), suspended and an aliquot was counted in an inverted microscope.

Meio- and macrofauna
Meiofauna organisms were extracted by a decantation method according to Noldt & Wehrenberg (1984). The sediment samples were in sequence rinsed in sea water, a solution of $MgCl_2$ and fresh water. The supernatant was poured through a set of sieves with mesh sizes of 250, 80 and 40 µm. Samples were preserved in 4 % carbonate buffered formalin containing Rose Bengal. Only samples for qualitative and quantitative analysis of turbellarians were treated without preservation. The organisms were sorted into main taxa and counted using a dissection microscope. Turbellarians were identified to species level and grouped into feeding types. Samples for macrofauna were sieved through 500 µm mesh size and live organisms were counted and identified to species level.

Statistical analyses
To get parallel samples and 'procedural treatment control samples' according to Hurlbert (1984), 12 PWMs were used. Six of them were randomly chosen to be filled with enrichment solution, the remaining six served as controls and were filled with filtered sea water. The measured variables were not normally distributed. Therefore the non-parametric *U*-test, according to Wilcoxon, Mann & Whitney (Sachs 1984), was used to test differences between treatments (one-way for nutrients and microphytobenthos, two-way for organic content and fauna). Differences were accepted as significant effects of treatment with $p < 0.05$.

Results

Inorganic nutrients
The concentrations of the dissolved inorganic nutrients PO_4^{3-}, NH_4^+, NO_2^- and NO_3^- in the pore water were increased by means of the PWMs (Figure 3). Phosphate concentrations in the controls were quite high and showed moderate fluctuations (3.1-16.8 µmol · dm^{-3}) during the experiment (Figure 3A). The addition of the enrichment solution did not result in an immediate increase in dissolved phosphate. After day 37, phosphate showed significant increases up to the 10-fold concentration in enriched plots (15.8-34.3 µmol · dm^{-3}). Ammonium occurred in relatively low concentrations in the controls (11.0-51.6 µmol · dm^{-3}) and was increased by the addition of the enrichment solution to very high values (145.1-745.0 µmol · dm^{-3}; Figure 3B). Nitrite and nitrate concentrations were always very low in controls (Figure 3 C&D). Although nitrate was the main N-component in the enrichment solution (8168 µmol · dm^{-3}), only little nitrate was found in the enriched pore water (15.2-76.2 µmol · dm^{-3}). For ammonium, the opposite situation was observed. Silicate behaved as expected with no significant differences between treatments (Figure 3A; day 37 is an exception). Besides this, concentrations were high and showed only moderate fluctuations

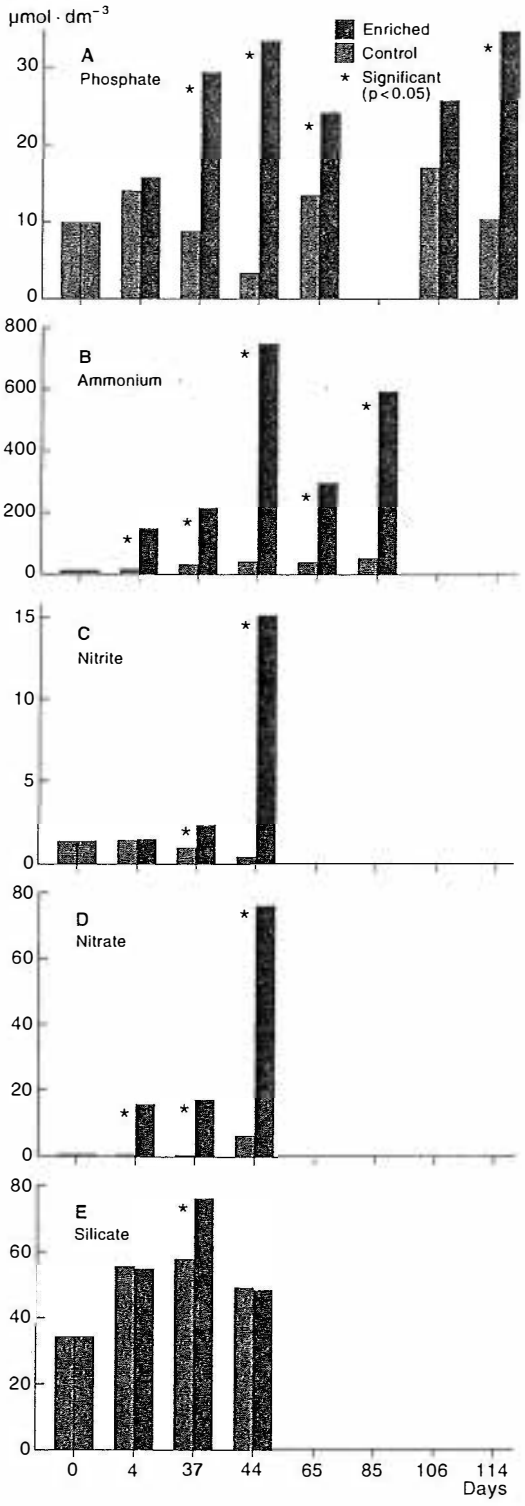

Figure 3.
Pore-water concentration of the dissolved inorganic nutrients. Each bar shows mean (n = 6).

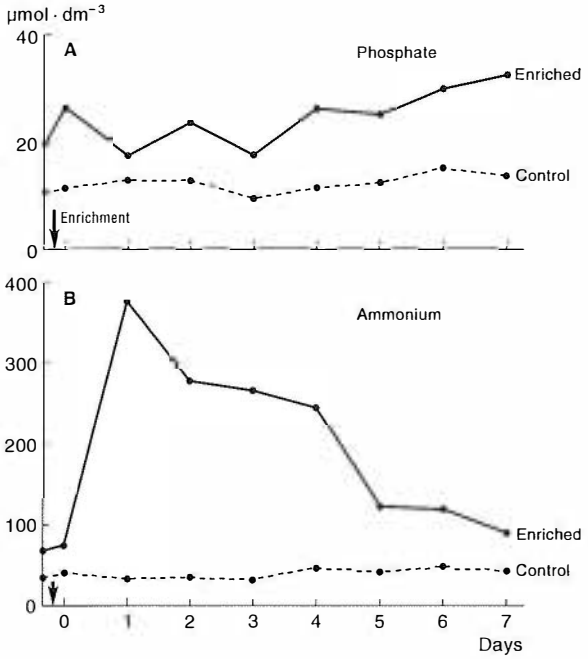

Figure 4.
Pore-water concentrations of phosphate (A) and ammonium (B) during one week. Curves are based on means of 6 replicates.

(34.2-57.4 µmol · dm^{-3}). The daily analyses of the concentrations of phosphate and ammonium during one week demonstrated the different behaviour of the two nutrients (Figure 4). Whereas the increase of phosphate remained quite uniform (about twice the concentration of the control (Figure 4A)), ammonium showed very strong fluctuations (Figure 4B). Immediately after enrichment, ammonium increased to the 11-fold concentration compared to controls. During the course of the week, it decreased to the 2-fold value. The concentrations of both nutrients in the controls did not show any reaction to the addition of filtered sea water. Analyses of data plots according to the 'mean-crowding' method (Lloyd 1967) indicated that the spatial distribution of phosphate and ammonium as well as of the N/P ratio was much more patchy in enriched plots than in controls. Because of the considerable increase in the ammonium concentrations, the N/P ratio was enhanced in the pore water in enriched plots (Table 1). It reached, thereby, mean values of 10, compared to 1-4 in controls. Thus, the experimental enrichment produced the optimum N/P ratio for algal growth. The proportion of silicate however was lowered by the addition of the enrichment solution, due to its very low silicate concentration.

	P	N	Si
Working value for diatoms*		10	10
Enrichment solution		9	0
Enriched pore water		10	4
Control pore water		1-4	4-7

*after Ryther & Dunstan (1971) and Doering *et al.* (1989).

Table 1.
Nutrient ratios.

Organic content

The organic content of the sediment did not show any significant difference between treatments (after 95 days). Highest values were found in the uppermost 0.5 cm (0.62 %). In the lower layers, it decreased to 0.33 %.

Visual observations

The sediment surface on enriched plots showed a more intense colour than on controls: greenish most of the summer and reddish-brown in autumn. Microscopic inspection revealed cyanobacteria causing the green and epipelic diatoms causing the red-brown colour. To build up a bloom, the microphytobenthos needed 3-4 days without strong winds resulting in water movements, as these destroyed the blooms through resuspension. Because of frequent storms during the experiment, no bloom on the enriched plots lasted longer than 10 days.

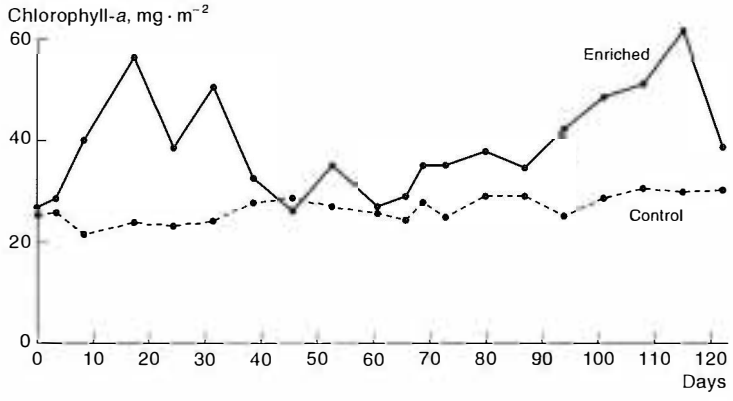

Figure 5.
Chlorophyll-*a* content of the sediment. Curves are based on means of 6 replicates. Samples from the upper 1 cm.

Chlorophyll-a

The mean chlorophyll-*a* content in controls varied between 21 and 31 mg · m^{-2}, in enriched plots between 26 and 62 mg · m^{-2} (Figure 5). There was a significant difference between treatments in most of the data plots. The increase of chlorophyll-*a* in enriched plots varied between +20 % and +139 %. The chlorophyll-*a* content showed strong fluctuations in enriched plots, due to the development and resuspension of blooms.

Cell numbers of autotrophs

Cyanobacteria. This group showed the most intense reaction to the enrichment (Figure 6A). At the beginning of the experiment, they occurred in low numbers (0.3×10^6 cells $\cdot$ cm^{-2}). However, during the course of the summer there developed several pronounced blooms (days 34, 55 and 95) in enriched plots with mean numbers of 12-14 $\times 10^6$ cells $\cdot$ cm^{-2}. This implied increases of 250-500% compared to controls (1-4 $\times 10^6$ cells $\cdot$ cm^{-2}). The blooms mainly consisted of the plate-shaped species *Merismopedia* cf. *elegans* A. Braun and *Merismopedia glauca* (Ehrenb.) Kütz., building quite large colonies.

Episammic diatoms. The episammic diatoms showed an increase during the course of the experiment from 2.7-4.2 $\times 10^6$ cells $\cdot$ cm^{-2} in controls and from 2.8-7.9 $\times 10^6$ cells $\cdot$ cm^{-2} in enriched plots (Figure 6B). There was a significant difference between treatments at day 10, 55 and 95, with increases of +70%, +20% and +87% in enriched plots.

Epipelic diatoms. In contrast to cyanobacteria and episammic diatoms, cell numbers of epipelic diatoms decreased in both the control and the enriched plots during the course of the summer from 21 and 30×10^3 cells $\cdot$ cm^{-2}, respectively to 5×10^3 cells $\cdot$ cm^{-2} (Figure 6C). There was a significant increase in enriched plots at day 10 and 55, but the most striking event occurred at day 95 (September) when a very heavy bloom developed in enriched plots: mean cell numbers of 160×10^3 cells $\cdot$ cm^{-2} implied an increase of more than 3000% compared to controls. This bloom was dominated by the genus *Nitzschia*. The epipelic diatoms are considered to be grazed primarily by the snail *Hydrobia ulvae*. The decrease of the epipelic diatoms during the course of the summer was accompanied by an increase of *H. ulvae* (Figure 9D). There were good inverse correlations found between abundances of epipelic diatoms and *H. ulvae* (Figure 7). In controls, with low numbers of diatoms, this relation was linear, whereas in enriched plots during a bloom (day 95), the relationship appeared exponential. Both the diatoms and the snails were distributed very patchily at that time.

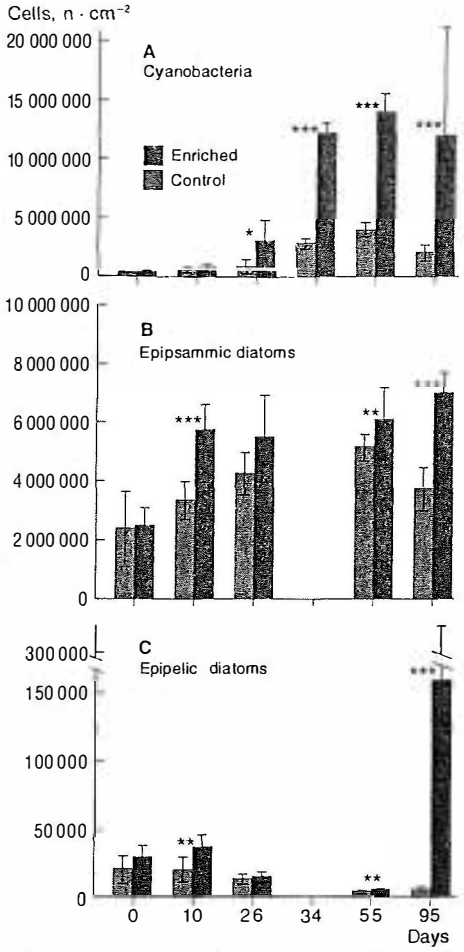

Figure 6.
Cell numbers of autotrophic microflora. Each bar shows mean ± SD (n = 6).
Significance: * = $p < 0.05$, ** = $p < 0.025$, *** = $p < 0.001$.

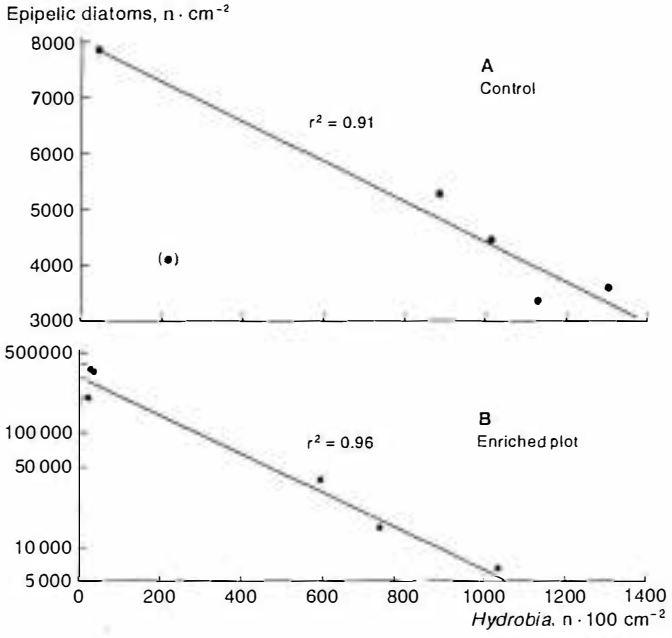

Figure 7.
Correlation of *Hydrobia ulvae* and epipelic diatoms. A: control; B: enriched plot.

Meiofauna

After 95 days, no significant increase in meiofauna could be found as a response to increased microphytobenthos. Abundances of nematodes were very high (10.0-15.2 $\times 10^3 \cdot 100\ cm^{-2}$) and almost identical in controls and enriched plots (Figure 8A). Harpacticoid copepods occurred in numbers of 1.7-7.1 $\times 10^3 \cdot 100\ cm^{-2}$ and showed even lower abundances in enriched plots (Figure 8B). In numbers of turbellarians, no significant difference between treatments could be found, neither for turbellarians as a whole (Figure 8C), nor for diatom-feeding species (Figure 8D). This group was not very abundant (6-67 $\cdot 100\ cm^{-2}$) and the data show high variances.

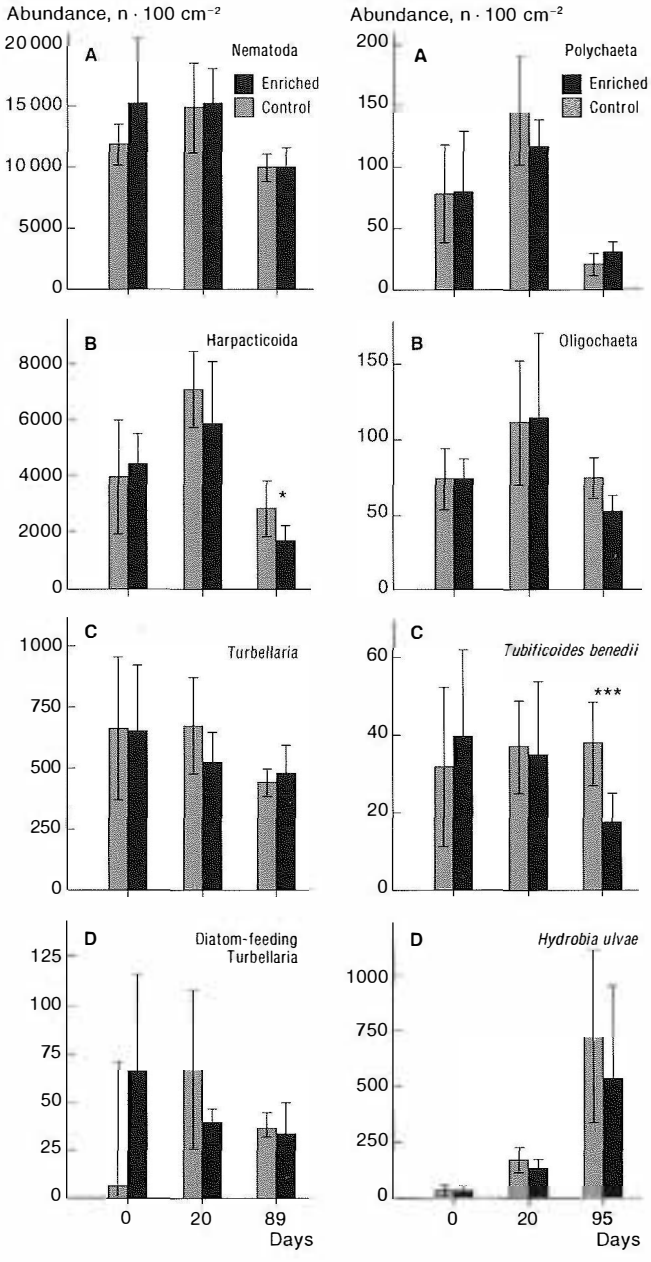

Figure 8. (left)
Abundances of the main meiofauna groups. Each bar shows mean ± SD (n = 6).

Figure 9. (right)
Abundances of the main macrofauna groups. Each bar shows mean ± SD (n = 6).
Significance: *** = $p < 0.001$.

Macrofauna

As for the meiofauna, no clear trend could be detected in macrofauna abundances, neither at the species level nor at any higher taxonomic group. Numbers of polychaetes, as the most diverse group, varied between 20 and 145 $\cdot 100\ cm^{-2}$ and showed no significant difference between treatments (Figure 9A). The same result applied for all other groups, e.g. oligochaetes as a whole (Figure 9B). Nevertheless, the very abundant species *Tubificoides benedii* occurred in significantly lower numbers at day 95 in enriched plots (Figure 9C). Abundance of the most important grazer, the snail *Hydrobia ulvae*, increased during the course of the summer in both controls and enriched plots (from 27 to 723 and from 26 to 532 $\cdot 100\ cm^{-2}$, respectively), but did not show any significant difference between treatments (Figure 9D).

Discussion

The pore-water manipulator

The PWM turned out to be a suitable device for an enrichment experiment. For the microphytobenthos, the sediment is the main source for nutrients (Vries & Hopstaken 1984), which are generated by remineralization of organic matter. Eutrophication of the North Sea also results in increased input of organic matter into the Wadden Sea and, thus, in elevated nutrient concentrations in the sediments (Helder 1974, Jonge & Postma 1974). This situation was successfully simulated by means of the PWM, providing the enrichment of the sediment from below. The experimental set-up allowed to repeat the enrichment and to use an enrichment solution of known composition and concentration. Sediment structure and community remained undisturbed during the experiment; the experimental plots were situated in the field without any delimitation to the natural environment. Comparisons with data plots from sediments without PWM indicated a natural structure of the experimental plots.

Fate of nutrients

Only a small portion of the injected nutrients could be detected in the pore water. It is most likely that, in a complex and dynamic system like an intertidal sediment, the nutrients undergo physical, chemical and biological processes that are very variable in space and time. This is also indicated by the patchy distribution. Phosphate is known to be removed from the dissolved phase by adsorption to sediment particles, even in an anoxic milieu (Schlungbaum 1982). The small fluctuations in the phosphate concentrations indicate a balance between adsorption and desorption. Ammonium is lost mainly by flux into the water column. This could be measured by means of bell-jars (Asmus 1986; own observations) and explains the decline of the ammonium concentration after an enrichment (Figure 4B). To introduce high concentrations of nitrate into anoxic sediment layers does not reflect the natural situation and was, therefore, a weak point of the experiment, that could have been avoided. Since it could not be found in the pore water, this nitrate is believed to have undergone reduction processes and have been used by denitrifying bacteria.

Reaction of microphytobenthos to nutrient enrichment

The sediment is often considered to be an inexhaustible source of nutrients so that a nutrient limitation for the microphytobenthos is excluded (Cadée & Hegeman 1974, Admiraal *et al.* 1982). On the other hand, there are many indications from field investigations and experiments suggesting positive reactions of microphytobenthos to increased nutrient concentrations, e.g. in salt marshes (Wiltse *et al.* 1984, Darley *et al.* 1981), shallow sublittoral sediments (Sundbäck & Jönsson 1988, Sundbäck & Granéli 1988) and also in the Wadden Sea (Otte 1979, Höpner & Wonneberger 1985). In the present study, the microphytobenthos biomass was enhanced by nutrient enrichment, although there were considerable concentrations of nutrients measurable in the pore water. It must, however, be kept in mind that concentration measurements were carried out in 4 cm depth whereas the primary production is limited to the uppermost millimeters; until now, it is practically impossible to record nutrient profiles on this scale. Concerning the nutrient ratios, nitrogen appeared to be the limiting nutrient (Table 1). Höpner & Wonneberger (1985) found the highest primary productivity where the N/P ratio was about 10 in the flux by diffusion out of an intertidal sediment, but normally it reaches 4 at maximum. The enhanced N/P ratio in enriched plots was accompanied by increased algal biomass. The organisms profiting most from the enrichment were cyanobacteria of the genus *Merismopedia*, well-known in the Wadden Sea to build up blooms which are related to eutrophication (Michaelis 1978, Colijn & Nienhuis 1978). This genus is not able to fix N_2 (Kapp *et al.* 1975) and is, therefore, dependent on other inorganic N sources. *Merismopedia* has a number of advantages compared to diatoms but two aspects are considered to be the most important in this case. Firstly, the cyanobacteria have a much higher division rate, namely 2-10 divisions per day (Bonin *et al.* 1982) whereas benthic diatoms are believed to divide only 0.3 times a day (Admiraal *et al.* 1982). Secondly, the cyanobacteria are hardly grazed (Nicotri 1977, Lee *et al.* 1985) as against epipelic diatoms which seem to be controlled by grazing (Connor *et al.* 1982, Asmus 1984). The development of the diatom bloom on day 95 was enabled by the extremely patchy distribution of the main grazer *Hydrobia ulvae* which occurred partly in very low numbers on enriched plots (Figure 7).

Reaction of zoobenthos to increased microphytobenthos

Although reported from similar experiments in salt marshes (Wiltse *et al.* 1984, K.H. Foreman & I. Valiela, pers. comm.), there was no reaction of meio- and macrofauna found in the present study. This means either that the fauna is not limited by food in this area, or that the enhanced microphytobenthos groups did not represent the right food for the investigated fauna. The microphytobenthos seems to contribute only a small amount to the organic matter in the sediment (Sundbäck *et al.* 1990). On day 95, during a heavy bloom of microalgae in enriched plots, there was no difference determined in the organic content between treatments. Because of resuspension of blooms, an accumulation of organic matter did not occur. Thus, there was no improved food availability for detritivores and omnivores. For grazers, the quality of food appears to be important. It has already been mentioned that cyanobacteria are hardly consumed. Only very few grazers are able to feed on the small, firmly attached episammic diatoms (Asmus 1984, Swamikannu & Hoagland 1989). For the epipelic diatoms, it is supposed that they suffer heavy grazing pressure by *Hydrobia ulvae*. In high numbers, such as those developing during the course of this experiment, the snails decimate their food to such an extent that their own growth can be impaired (Fenchel & Kofoed 1976, Levinton 1985). This means for other diatom grazers that there did not occur a dramatically improved food situation. For the macrofauna in general, the studied period might be too short to find clear correlations.

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